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MODULATION OF ACETYLATION OF CHROMOSOMAL PROTEINS OF THE BRAIN OF RATS OF VARIOUS AGES BY EPINEPHRINE AND ESTRADIOL

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ABSTRACT - In vitro acetylation of histones and non histone chromosomal proteins (NHCP) and their modulation by epinephrine and estradiol were studied by incubating slices of cerebral hemisphere of young (2-), adult (15-) and old (84-week) female rats with ^{14}C-sodium acetate. Acetylation of histones decreases with increasing age. Both epinephrine and estradiol stimulate acetylation in the young. Stimulation by epinephrine is greatly decreased in the adult, and it has no effect in the old. Estradiol has no effect in adult and old rats. Acetylation of H3, H4 and H2B histones decreases with age, but not of H1 and H2A. Epinephrine and estradiol significantly stimulate acetylation of H1 and H3 respectively in the young. Acetylation of NHCP is high in the young and decreases precipitously with increasing age. Both epinephrine and estradiol stimulate acetylation of NHCP in young, but not in adult and old rats. Such age-related alterations in acetylation of chromosomal proteins by hormones and other effectors may be responsible for differential activation and repression of genes and cause aging.

INTRODUCTION

Histones and non-histone chromosomal proteins (NHCP) are involved in the regulation of gene activity. Their effects are brought about by modifications such as phosphorylation, acetylation and methylation which alter their association with DNA. Hormones modulate such modifications, and thereby alter gene expression (1). We recently reported for the first time that phosphorylation of histones of the brain of rats decreases, and that of NHCP increases as a function of age (2). Calcium inhibits phosphorylation of specific histones, but stimulates that of NHCP. Histone acetylation is reported to coincide with an increase in transcription (3). We report here that

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Abbreviation : NHCP, non-histone chromosomal proteins.

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Table 1. In vitro incorporation of ^{14}C-acetate into total histone and non-histone chromosomal proteins of cerebral hemisphere of female rats of various ages, and effects of L-epinephrine ($10^{-5}M$) and 17B-estradiol ($10^{-6}M$) on their acetylation.

Age (weeks)	Experimental Condition	Histone		NHCP	
		CPM/g wet wt.	Sp. activity (CPM/μg histone)	CPM/g wet wt.	Sp. activity (CPM/μg NHCP)
2	Slice + AC^-	3741	0.94	8293	2.20
	Slice + Ep + AC^-	6402	1.79	9505	3.58
	Slice + Est + AC^-	4131	1.35	9158	3.18
15	Slice + AC^-	1581	0.70	543	0.28
	Slice + Ep + AC^-	1993	0.86	961	0.37
	Slice + Est + AC^-	2581	0.73	877	0.31
84	Slice + AC^-	1505	0.55	226	0.12
	Slice + Ep + AC^-	1612	0.59	191	0.11
	Slice + Est + AC^-	1440	0.53	297	0.13

acetylation of both histones and NHCP of the brain of rats decreases with age, and epinephrine and estradiol modulate acetylation of NHCP and specific histones.

MATERIALS AND METHODS

Young (2-), adult (15-) and old (84-week) female rats of Wistar strain maintained under standard conditions were used. Cerebral hemispheres were removed and immediately cut into slices of approximately 0.4mm thickness. In vitro acetylation of chromosomal proteins was studied according to the method of Sung et al (4). 1.0g of the sliced tissue was incubated in each flask containing 4.0 ml Krebs-Ringer bicarbonate buffer, pH 7.4. Epinephrine (10.0 μ mole) or 17-βestradiol (1.0 μ mole) was added to experimental flasks. Cycloheximide ($2 \times 10^{-4}M$) was added to each flask to inhibit protein synthesis. The control and experimental flasks were set up in duplicate. The flasks were then shaken for 30 min. in a water bath maintained at 37°C. Then 0.1 mCi of (U-^{14}C) sodium acetate (Bhabha Atomic Research Centre, Trombay, India) was added to control and experimental flasks and shaking was continued for 60 min. The slices were then taken out and washed thrice in cold Krebs-Ringer bicarbonate buffer to remove ^{14}C-acetate.

The extraction of histones and NHCP from the chromatin, and fractionation of individual histones were done according to Bonner et al (5) and Elgin and Bonner (6), and as adopted by Kanungo and Thakur (2). The radioactivity was counted in an LS-100C Beckman Scintillation system.

RESULTS AND DISCUSSION

Table 1 shows that acetylation of histones of the brain rapidly decreases till adulthood, and does not change thereafter. Furthermore, acetylation of individual histones varies with age (Table 2). Whereas acetylation of three

Table 2. In vitro incorporation of ^{14}C-acetate into individual histones (CPM) of the cerebral hemisphere of rats of three ages and effects of epinephrine and estradiol on acetylation.

Age (weeks)		Slice + AC^-	Slice + Ep + AC^-	Slice + Est + AC^-
2	H1	62	145	82
	H2A	138	140	164
	H2B	195	180	174
	H3	85	72	178
	H4	90	98	80
15	H1	65	85	78
	H2A	130	148	155
	H2B	160	170	163
	H3	72	70	88
	H4	65	98	76
84	H1	65	62	58
	H2A	130	170	154
	H2B	90	90	124
	H3	60	35	68
	H4	44	62	50

nucleosomal histones, H4, H3 and H2B, is high in the immature and decreases with age, that of H1 does not show any age-related change. The neurons of the brain of the rat stop dividing after early development. The significant decrease in the acetylation of histones, particularly of the nucleosome, after early development may be due to cessation of neuronal division. Also, a decrease in the activity of histone acetyltransferase and/or the availability of acetylation sites, particularly of lysyl residues, due to conformational changes in the DNA-histone complex after the cells stop dividing, may account for the decrease in acetylation of histones.

Both epinephrine and estradiol significantly stimulate acetylation of histones in immature rats. The stimulatory effect of epinephrine is greatly reduced in the adult, and is not apparent in the old. Estradiol has no effect in the adult and the old. Furthermore, epinephrine stimulates acetylation of H1 in the immature significantly, but slightly in the adult. Estradiol, on the other hand, stimulates acetylation of H3 only in the immature. Such differences in the modulation of acetylation by epinephrine and estradiol are of particular significance as the mode of action of the two hormones are

different; the former initiating effects at the cell membrane, and the latter at the level of the chromatin through a cytosol-receptor.

Acetylation of NHCP is high in the immature and decreases precipitously thereafter (Table 1). This decrease is far greater than that for histones. Both epinephrine and estradiol stimulate acetylation of NHCP in the immature significantly. NHCP constitute a large number and variety of proteins, and they are believed to be involved in specific regulation of genes (7,8). It may, therefore, be of interest to find out if the acetylation of specific NHCP is altered with age, and if epinephrine and estradiol modulate acetylation of specific NHCP.

These data together with our earlier findings that phosphorylation of NHCP and specific histones is altered with increasing age of rats (2) for the first time give insight into the functional changes that occur at the level of the genome during aging. Differential effects of hormones and other factors, whose levels change during the life span and under various physiological conditions, at the level of the chromatin may be responsible for selective activation and repression of genes and account for the changes in levels of enzymes and other proteins as a function of age of an organism. This is consistent with the model for aging proposed by Kanungo (9) according to which sequential changes in activities of genes brought about by various factors may cause aging.

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References

1. Langan, T.A. (1969) Proc. Natl. Acad. Sci. (USA), 64, 1276.
2. Kanungo, M.S. & Thakur, M.K. (1977) Biochem. Biophys. Res. Commun. 79, 1031.
3. Alefrey, V.G. (1971) in Histones and Nucleohistones (Phillips, D.M.P. Ed.) pp. 241, Plenum Press, NY.
4. Sung, M.T., Harford, J., Bundman, M. & Vidalakas, G. (1977) Biochemistry, 16, 279.
5. Bonner, J. et al (1968) Meth. Enzymol. 12B:3.
6. Elgin, S.C.R. & Bonner, Jr. (1970) Biochemistry, 9, 4440.
7. Spelsberg, T.C., Wilhelm, J.A. & Hnilica, L.S. (1972) Sub-Cell. Biochem. 1, 107.
8. Ruiz-Carillo, A., Wanga, L.J. & Allfrey, V.G. (1976) Arch. Biochem. Biophys. 174, 273.
9. Kanungo, M.S. (1975) J. Theor. Biol., 53, 253.